both. Oxidatives stress was induced by pre-treatment with 250uM tert-butyl hydroperoxide (TBHP). DNA was measured with a picogreen assay, PG synthesis by sulfate incorporation, survival with the Live-Dead cell assay and cell signaling by immunoblotting with phospho-specific and control antibodies. Smad 4 translocation to the nucleus was measured by confocal microscopy.

Results: With increasing donor age, chondrocytes had a reduced response (PG synthesis corrected for DNA) to IGF-1 ($r=-0.63$, $p=0.02$), IGF-1+OP-1 ($r=-0.74$, $p=0.004$) and a trend for OP-1 alone ($r=-0.51$, $p=0.08$). In 21-day alginate bead cultures there was a significant reduction in DNA content with age in controls ($r=-0.57$, $p=0.002$) and although IGF-1, OP-1, and IGF-1+OP-1 increased DNA content over controls, there was a similar age-related decline in DNA with all treatments. IGF-1 activated both the PI-3 kinase-Akt and MEK-ERK pathways while OP-1 activated Smad1,5,8 and Smad 4 translocation. DNA content in 21-day alginate beads was significantly reduced with all treatments in the presence of the PI-3 kinase inhibitor LY294002 but not with the MEK inhibitor PD98059 consistent with a requirement for Akt activation to promote chondrocyte proliferation and survival. Induction of oxidatives stress with TBHP inhibited IGF-1 activation of Akt but not ERK which was activated. Cells from older adults had significantly greater ERK activation with TBHP ($p=0.04$) and greater Akt inhibition ($p=0.002$) than cells from younger donors demonstrating greater susceptibility to oxidative stress (Fig. 1). TBHP did not appear to affect Smad1,5,8 phosphorylation in response to OP-1 and only modestly reduced the amount of Smad 4 translocation to the nucleus by about 20% but completely inhibited OP-1 induced PG synthesis to levels below control.

Conclusions: Both increasing age and oxidative stress reduce the anabolic and survival response of normal human chondrocytes to IGF-1 and OP-1. The PI-3kinase-Akt pathway is required for survival and proliferation of chondrocytes in response to both IGF-1 and OP-1 even though only IGF-1 directly activates this pathway. Although oxidative stress inhibited OP-1 stimulation of PG synthesis it did not appear to significantly inhibit Smad signaling suggesting other pathways are involved. In previous work we have shown OP-1 promotes IGF-1 signaling by increasing IGF receptor expression and so the effects of oxidative stress on the response to OP-1 may be due to inhibition of the IGF-1 signaling rather than directly inhibiting OP-1 pathways.

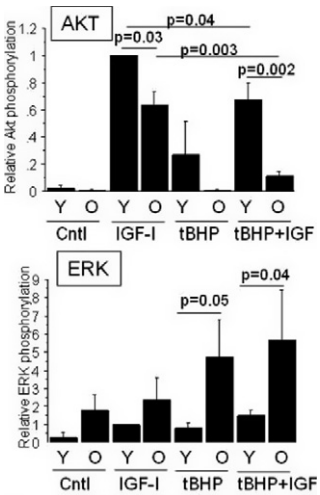

Fig. 1. Phosphorylation of Akt and ERK in normal chondrocytes from young (Y) and old (O) donors.

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INFLUENCE OF THE DIFFERENCE IN REPETITION FREQUENCY AND INTENSITY OF LOW INTENSITY PULSED ULTRASOUND FOR CULTURED CHONDROCYTES

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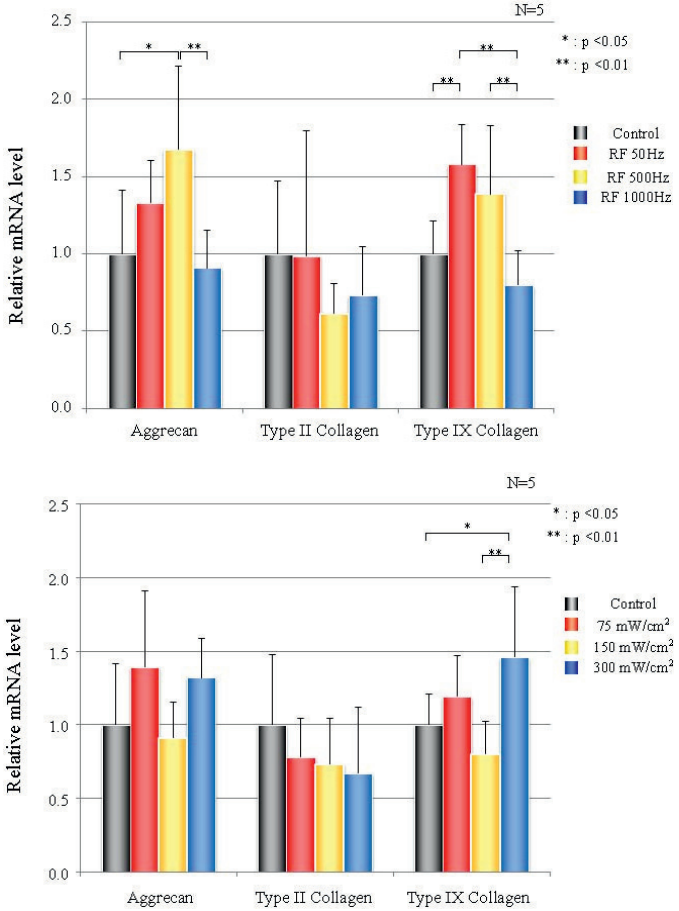
Purpose: The purpose of this study is to clarify the influence of changing repetition frequency(RF) and intensity of Low-Intensity Pulsed Ultrasound (LIPUS) for cultured bovine articular chondrocytes.

Methods: Chondrocytes were obtained from the metatarso-phalangeal joints of freshly slaughtered 30 months old bovine. Cells were cultured on 6-well plates and the final cell density was adjusted to 3.3×10^5 cells/well/ml. At first, to investigate the effect of RF, intensity of LIPUS was set to 150 mW/cm^2|SATP constantly. LIPUS was exposed at RF of 50, 500, and 1000 Hz. The second, to investigate the effect of intensity, RF was set to 1000 Hz. LIPUS was exposed at intensity of 75, 150, and 300 mW/cm^2|SATP. LIPUS stimulation (frequency of 1.5MHz and burst duration of 200 μs) for 20 min every day was applied to chondrocytes after 24 hours in culture through the bottom of the culture dish via water between the LIPUS transducer and the dish. At the time of 7days after culture, mRNA levels of Aggrecan (AGC), Type II collagen (COL2), and Type IX collagen (COL9) were evaluated by Real-time RT-PCR.



Results: In intensity was 150 mW/cm^2|SATP and RF was changed, the RF of 500 Hz was found to increase expression of mRNA for AGC by 168% of control ($p<0.05$). The RF of 50 Hz was found to increase expression of mRNA for COL9 by 158% of control ($p<0.01$). In RF was set to 1000 Hz and intensity was changed, intensity of 300 mW/cm^2|SATP was found to increase expression of mRNA for COL9 by 147% of control ($p<0.05$).

Conclusions: These results suggested that changing of RF of LIPUS may affect expression of each mRNA. In addition, when RF was fixed, changing of intensity may affect to expression of only COL9 mRNA. The changing of intensity and RF of LIPUS may influence to some products produced by chondrocytes during culture.